
Aptamer-based biosensors
nanoschematic

Aptamer-based biosensors

- ***Qazvin university of medical science***

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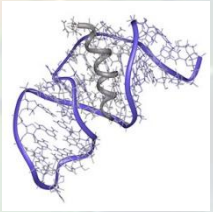
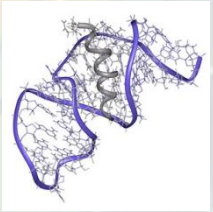


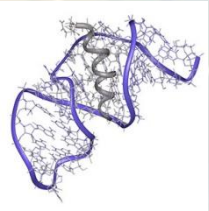


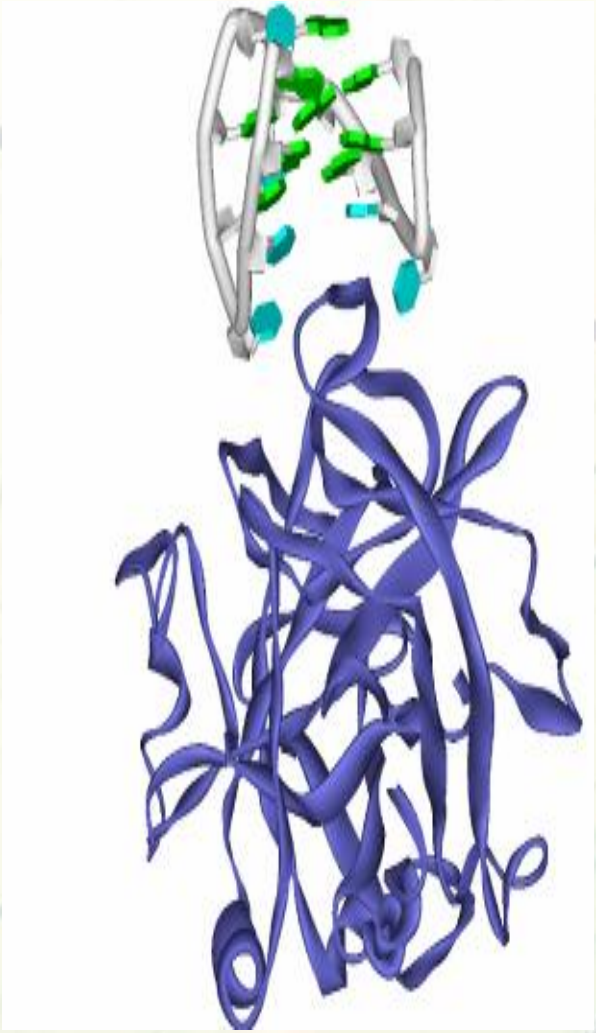
History

- Spiegeman and his colleagues publish their work with nucleic acid in 1960.

- The result was the invention of PCR in 1986 by Molis.

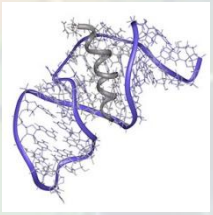
- In 1990 SELEX (Selective expansion of ligands by exponential enrichment) were invented.





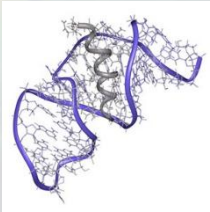
- ✓ **Aptamers (By Ellington) are oligonucleotides**
- ✓ **(DNA or RNA) that can bind with high affinity**
- ✓ **specificity to a wide range of target molecules such as drugs, proteins or other organic or in organic molecules**

Aptamers, derived from the Latin aptus,meaning ‘‘to fit”



- Each RNA molecules have specific sequences of nucleic acids.
- These molecules bind to different positions
- Establish hydrogen bonds and van der Waals molecules
- These RNA are called Aptamer and the binding site are named Aptatop.

Aptamers VS Antibodies



	Aptamers	Antibodies
Affinity	Low nM to pM	Low nM to pM
Specificity	High	High
Production	In Vitro Chemical Process	In Vivo Biological Systems
Target Range*	Wide: Ions, Cell, Toxins	Narrow: Immunogenics
Batch to Batch Variation	Little or No	Significant
Chemical Modification*	Easy	Limited
Thermal Denaturation	Reversible	Irreversible

Aptamers: Denser immobilization on the surface

Aptamers: Nuclease sensitive

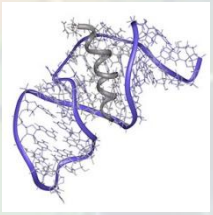
Creation of Aptamers

- Aptamers are generated by an iterative selection process, called systematic evolution of ligands by exponential enrichment (SELEX).

- SELEX technology have extended aptamer selection from comparatively simple mixtures of purified proteins to whole living cells.

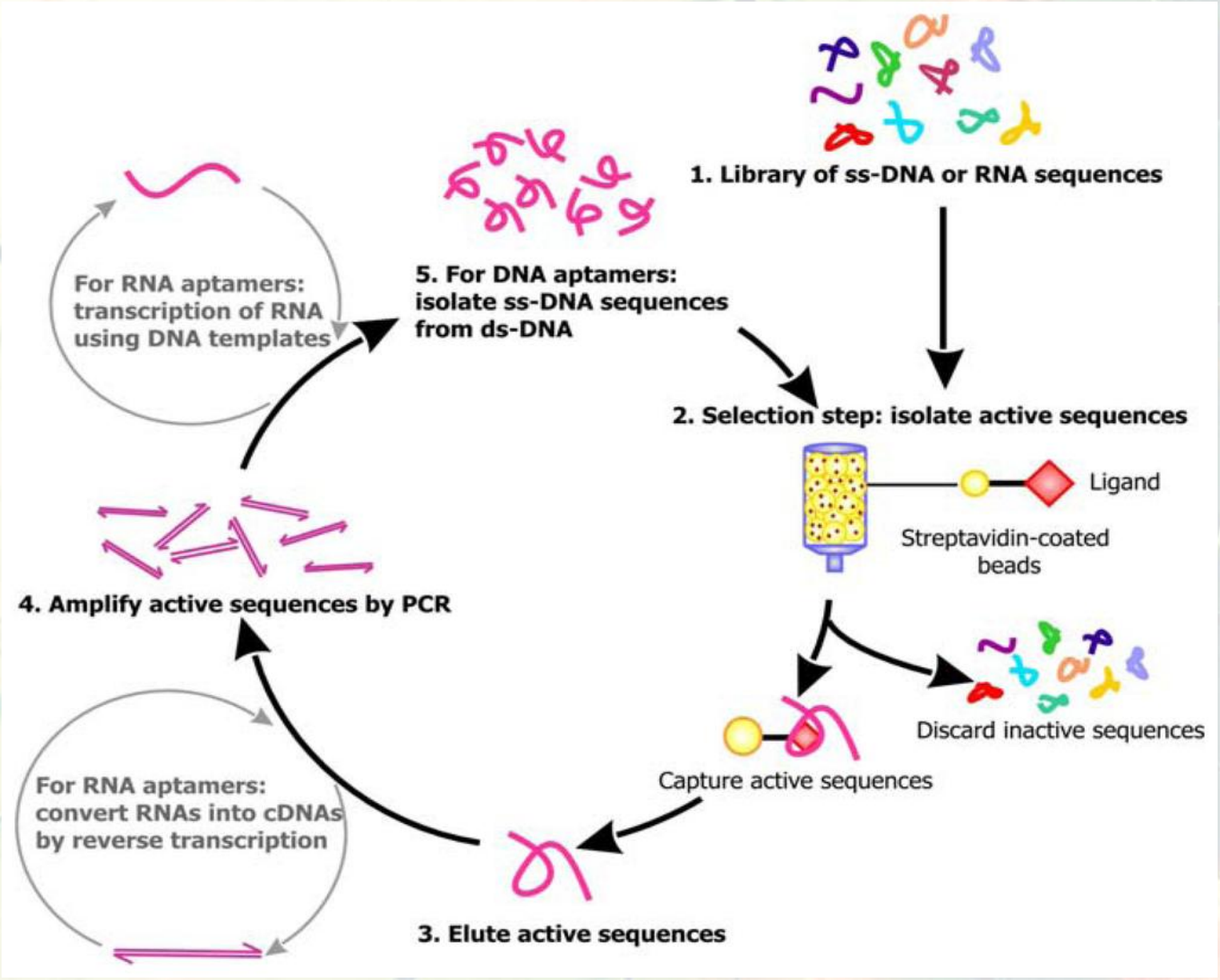
- SELEX isolate Aptamers that bind to specific target cells.

SELEX

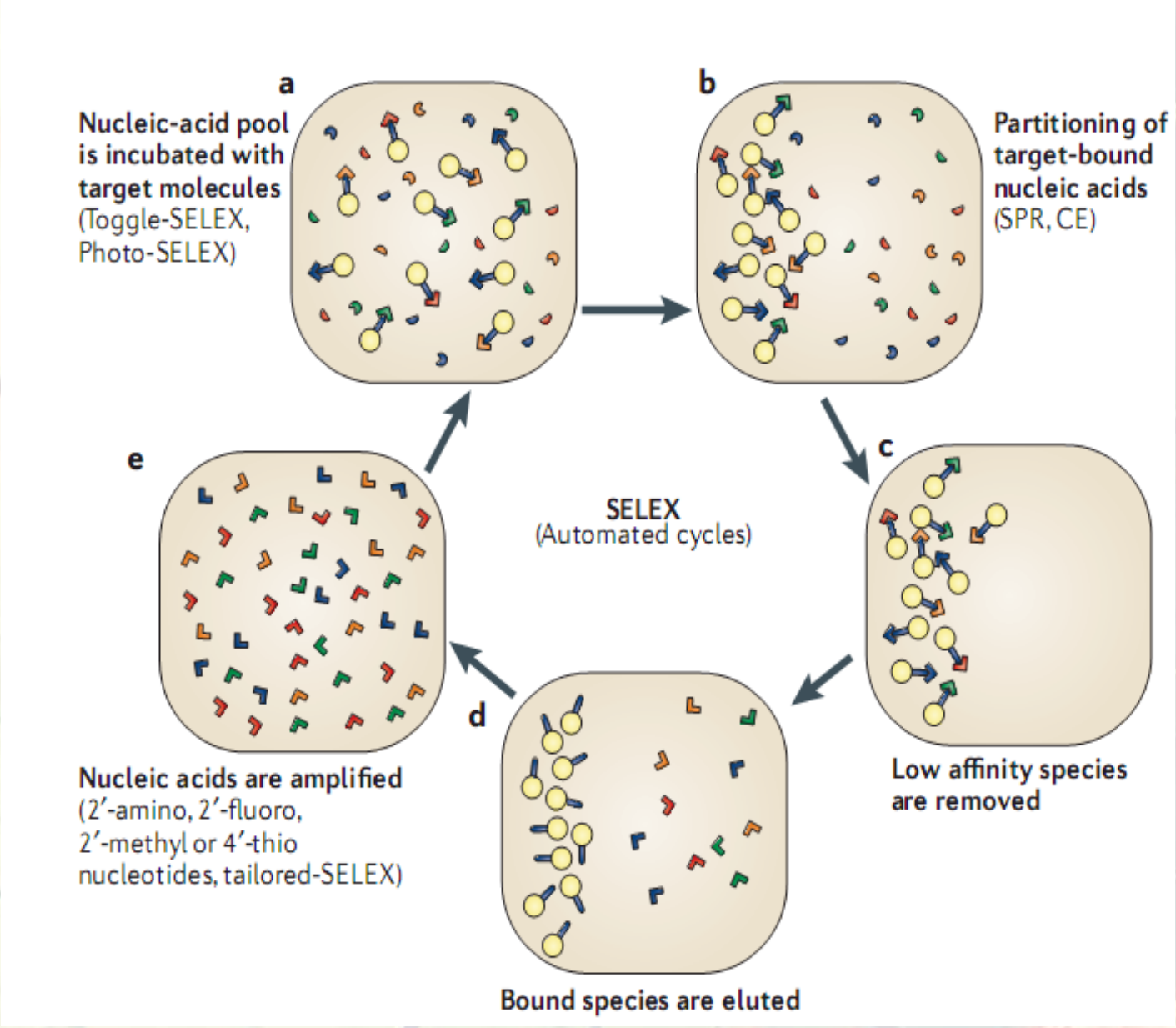


Systematic Evolution Of Ligands By EXponential Enrichment

- **SELEX is a multi-step process in which strongly bind**
- **ligands are preferably selected by rounds of affinity assays and PCR amplification**
- **The SELEX method has permitted the identification of unique RNA/DNA molecules which bind to the target with very high affinity and specificity**


1. Library of ss-DNA or RNA sequences
2. Selection step: isolate active sequences
Ligand
Streptavidin-coated beads
Discard inactive sequences
Capture active sequences
3. Elute active sequences
For RNA aptamers: convert RNAs into cDNAs by reverse transcription
4. Amplify active sequences by PCR
For RNA aptamers: transcription of RNA using DNA templates
5. For DNA aptamers: isolate ss-DNA sequences from ds-DNA

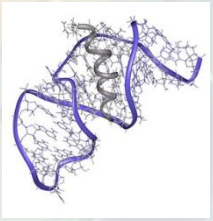
[2]


a
Nucleic-acid pool is incubated with target molecules (Toggle-SELEX, Photo-SELEX)
b
Partitioning of target-bound nucleic acids (SPR, CE)
c
Low affinity species are removed
d
Bound species are eluted
e
Nucleic acids are amplified (2′-amino, 2′-fluoro, 2′-methyl or 4′-thio nucleotides, tailored-SELEX)
SELEX
(Automated cycles)

[3]

circular dichroism spectroscopy

Bio sensors



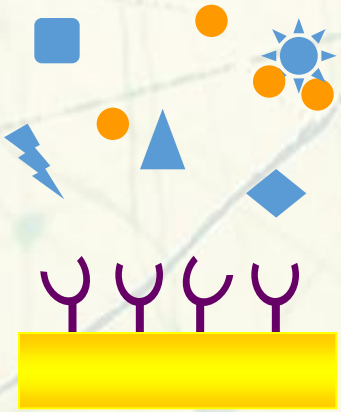
- A sensor is a device that transforms environmental information, ranging from the concentration of a specific sample component to total
- composition analysis, into an analytically useful signal.

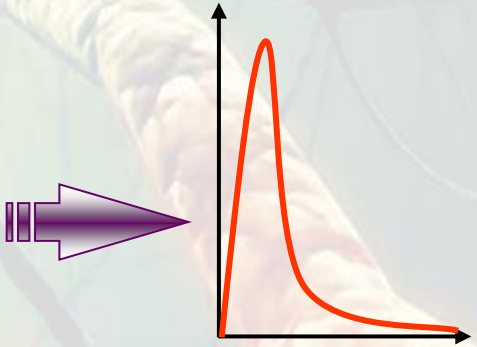
Recognition Part

- Antibody
- Enzyme
- Microorganism
- Aptamer

Transducer

- Electrochemical
  - potentiometric
  - amperometric
  - potentiometric stripping analysis
- Thermal
  - quartz crystal microbalance
  - surface acoustic wave
  - surface transverse wave
- Optical
  - absorbance
  - luminescence
  - fluorescence
  - total reflectance fluorescence
- Mass Changes



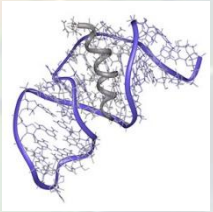


Receptor

Transducer

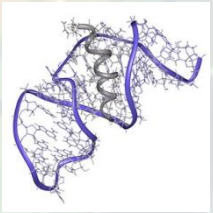
Recorder

Essential Factors for Biosensors Operation



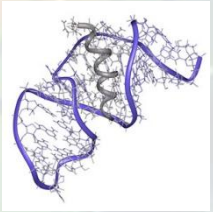
- *Sensitivity to presence of target*
  - The fabricated biosensor should show sensitivity to the presence of its own target.

- *Linearity of response*
  - The response of biosensor should be proportional to the concentration of its target.



- *Selectivity*
  - Chemicals interference must be minimized for obtaining the correct result.

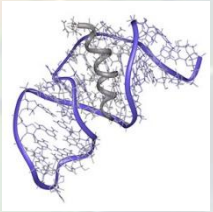
- *Reproducibility*
  - The response of the biosensor to the presence of target should be reproducible.



Detection of Aptamer

- **Using affinity surfaces**
- **Using affinity tags**
- **Using column matrices or ligands**
- **Nitrocellulose membrane filtration**
- **Cross-linking**
- **Antibody-based**
- **Surface plasmon resonance**
- **Capillary electrophoresis**
- **Flow cytometry**
- **Automated selection**

Using affinity surfaces



- **Affinity surfaces: allow proteins and small molecules to bind with them & have affinities with RNA or DNA.**

- **Magnetic beads, affinity titer plates**

- **RNA Aptamer against Panama influenza virus subtype A**

Step 1
Preparation

Co Amplification

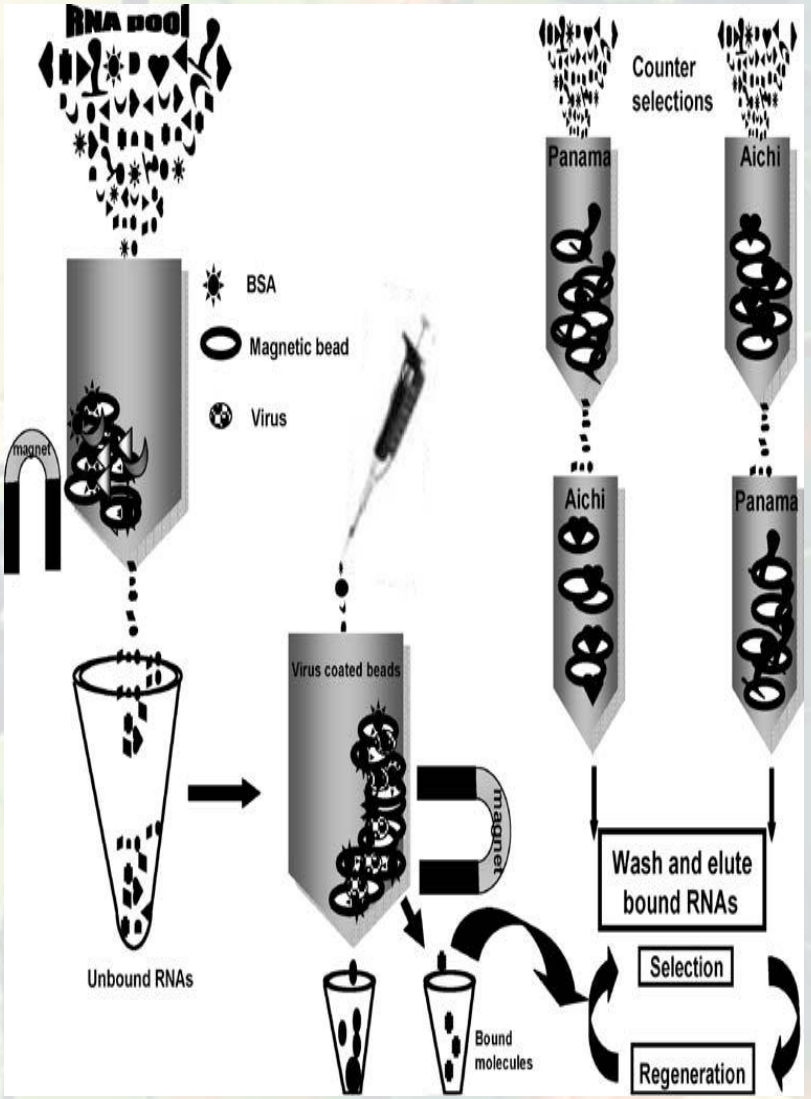
•Coating the whole virus onto beads

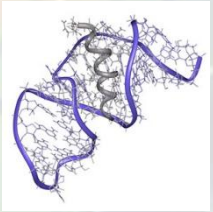
•BSA Blocking

•Washing the coated beads.

•Denaturing the pool RNA(90 ° C for 2 min and allowed to cool at room temperature for 10 min)

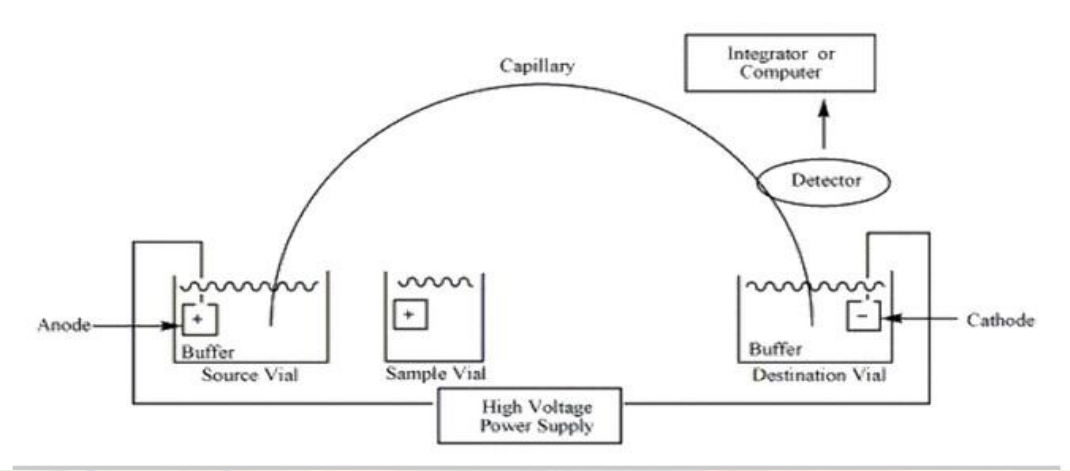
Bound molecules were precipitated by ethanol.

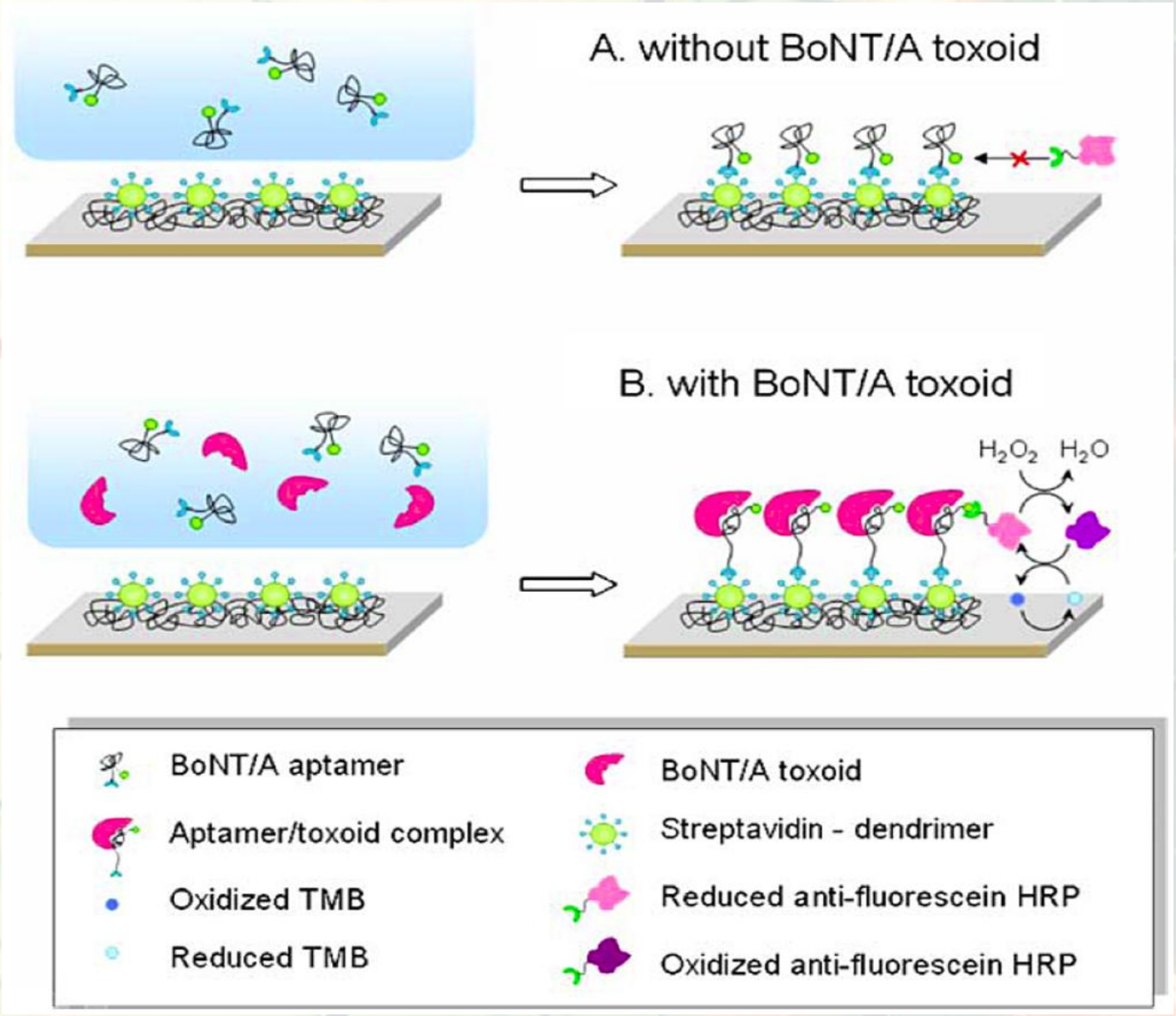




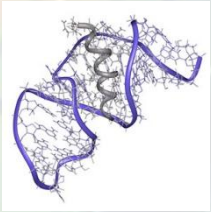
Using capillary electrophoresis

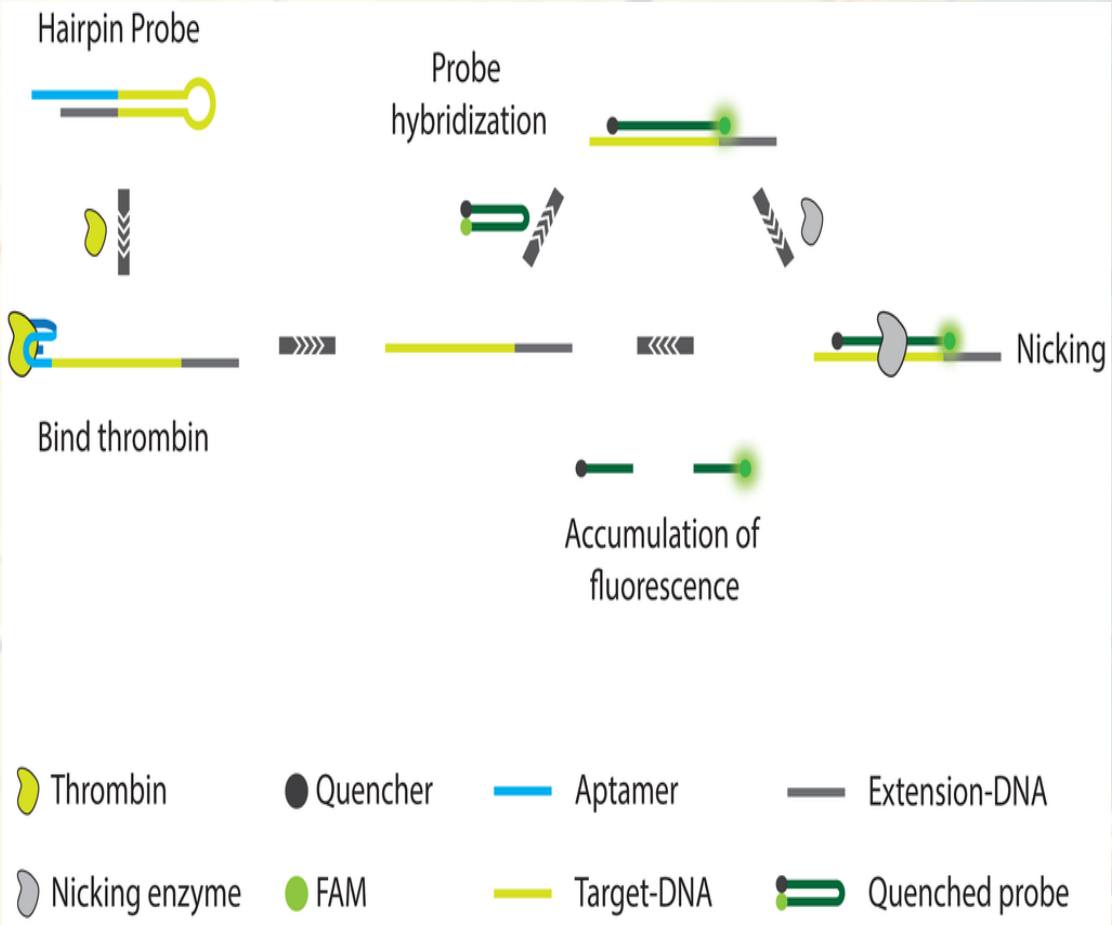
- **The nucleic acid sequences that bind the target undergo a mobility shift.**
- **no need to wash the active sequences off a column as in conventional SELEX, eliminating any kinetic bias.**
- **Higher speed, better resolution capacity, minimal sample dilution, fewer cycles.**



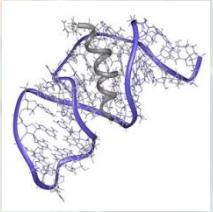

A. without BoNT/A toxoid
B. with BoNT/A toxoid
H_2O_2 H_2O
BoNT/A aptamer
Aptamer/toxoid complex
Oxidized TMB
Reduced TMB
BoNT/A toxoid
Streptavidin - dendrimer
Reduced anti-fluorescein HRP
Oxidized anti-fluorescein HRP

Fluorescent signals





Application



- Therapeutic Target
- Analytic Detection

APTAMER TO THERAPEUTIC TARGET

Target (alternative name)	K_d (nM)	Therapeutic applications
α-thrombin	25	Prevent thrombosis
HIV-1 reverse transcriptase	1	Inhibit viral replication
HIV-1 Rev	<1	Inhibit viral replication
Fibroblast growth factor 2, basic	0.35	Prevent angiogenesis
Respiratory syncytial virus	40	Prevent infection
HIV-1 integrase	10	Inhibit viral replication
Vascular endothelial growth factor	0.14	Prevent neovascularization
Platelet-derived growth factor	0.1	Prevent tumour development
Immunoglobulin E	10	Prevent allergies
L-Selectin	3	Modulate inflammation
D-Adenosine	1,100	Unknown
Acetylcholine-specific auto-antibodies	60	Treat myasthenia gravis
Interferon-γ	68	Modulate inflammation and immune response
Keratinocyte growth factor	0.0002	Treat epithelial hyperproliferative disease
Neutrophil elastase	n/o	Modulate inflammation
P-selectin	0.04	Inhibit viral adhesion
Acetylcholine receptor	2	Control neurotransmission
Phospholipase A_2	118	Treat ARDS, septic shock
Protein tyrosine phosphatase	18	Inhibit oncogenesis, viral regulation
Activated protein C	110	Prevent thrombosis
CD4	0.5	Modulate immune response
Nuclear factor-κB	1	Treat chronic inflammatory disease
Lymphocyte function-associated antigen 1	500	Prevent tumour development, modulate inflammation
Cytohesin 1	5	Modulate cytoskeletal reorganization
αvβ3 integrin	2	Prevent tumour development

APTAMER TO THERAPEUTIC TARGET

Target (alternative name)	K_d (nM)	Therapeutic applications
Tenascin C	4	Prevent tumour development
Prostate-specific membrane antigen	2.1	Treat progressive malignant prostate disease
U1A	4.5	Modulate gene regulation
Gonadotropin-releasing hormone 1	50	Prevent tumour development
E2F transcription factor	15	Prevent tumour development
Neurotensin 1	1.5	Prevent viral infection
Factor IXa	0.65	Prevent thrombosis
NS3 protease	10	Treat hepatitis C virus infection
Staphylococcus enterotoxin B	420	Treat *Staphylococcus* infection
Chemokine (C-C motif) ligand 2 (MCP1)	3	Treat lupus
Angiopoietin 2	0.06	Prevent angiogenesis
HIV gp120	5	Inhibit viral infectivity
Calcitonin gene-related peptide	3	Treat migraine
HER3 (ERBB3)	45	Prevent tumour development
Cytotoxic T-lymphocyte-associated protein 4	10	Prevent tumour development
Cytohesin 2	115	Prevent tumour development
Nociceptin	110	Manage pain
Ghrelin	35	Prevent tumour development
Chemokine (C-X-C motif) ligand 10 (IP-10)	1.5	Modulate inflammation
Receptor tyrosine kinase RETC634Y	35	Prevent tumour development
Substance P	40	Prevent tumour development
Mucin 1	0.135	Prevent tumour development
Amylin	3	Treat pancreatic cancer
Angiopoietin 1	2.8	Prevent angiogenesis
Bovine prion protein	6.8	Treat prion and Alzheimer's disease
Plasminogen activator inhibitor 1	n/o	Prevent metastasis
Epidermal growth factor receptor variant III	33	Prevent tumour development

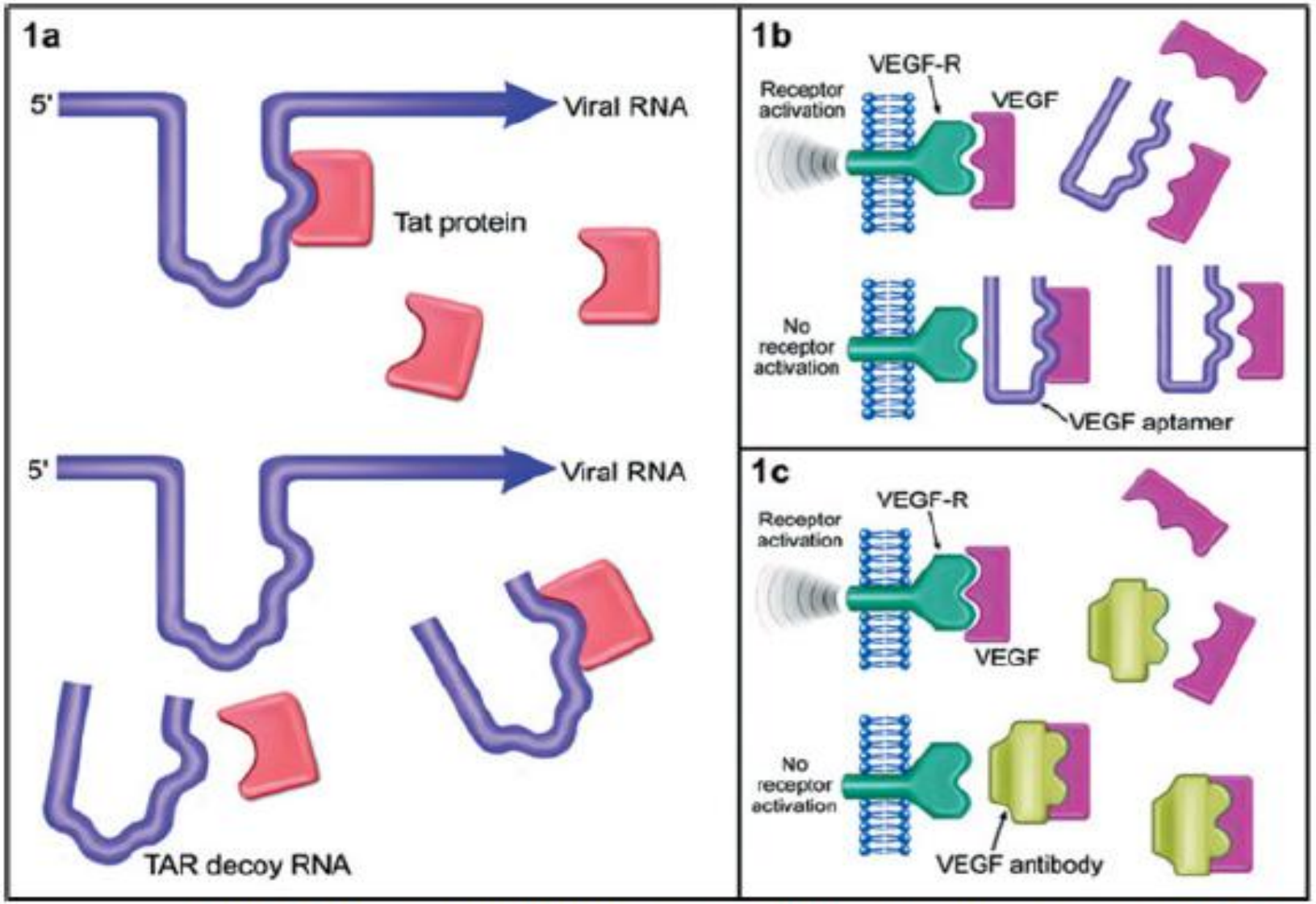


Fig. 6.1 Aptamer–protein interaction. (a) TAR decoy RNA-mediated inhibition of Tat. The TAR decoy RNA is similar to the endogenous viral TAR RNA and assumes an analogous tertiary structure and in so doing, competes with viral TAR for Tat binding. (b) Anti-VEGF aptamer inhibits VEGF receptor activation by VEGF by the same mechanism as (c) an antibody targeted to the same molecule. TAR. *trans*-activation response element; Tat, *trans*-activating regulatory protein; VEGF, Vascular endothelial growth factor.

Aptamer and Epidermal growth factor receptor

- Epidermal growth factor receptor (EGFR/HER1/c-ErbB1), is overexpressed in many solid cancers.
- TuTu22 aptamer was able to recognize cancer cells expressing EGFR but did not bind to the EGFR-negative cells.
- This DNA aptamers will facilitate the development of novel targeted tumor cell detection, cancer cell imaging and cancer therapy.

analytical applications

- **Affinity Chromatography**
- **Capillary Electrophoresis**
- ***In vitro* and *in vivo* diagnostic tools**
- **Targeting intracellular target molecules**
- **Drug Discovery**
- **Therapy**
- **Protein Purification**
- **Diagnostics**
- **ELISA**
- **Western Blotting**

Analytic Detection:Aptamers to Nucleic Acid

- ✓ The first aptamer to a nucleotide was isolated in 1993 by Sassanfar and Szostak

- ✓ In 2004 RNA selection aptamers that recognized the triphosphate of ATP were selected in the laboratory of Jack W. Szostak (Sazani et al., 2004)

- ✓ Interact with the part of the target molecule that faced the matrix, but did not strongly interact with the part that had greatest accessibility.

Conclusion

- Aptamer technology has made significant strides.
- Aptamers can be applied to many fields of molecular medicine
- The identification of aptamer molecular targets constitutes a novel method of biomarker discovery

- Cell-specific aptamers can be readily adapted for drug delivery and targeted therapy

- Cell-SELEX represents an integrated technology that will revolutionize the way we diagnose, treat and prevent disease

- represents an integrated technology that will revolutionize the way we diagnose, treat and revent disease.

Research Perspectives

- ✓Aptamers are the very astonish way to diagnose and diagnosis disease
- ✓It seems using Aptamer will help us one step closer to understanding all sort of sickness especially in cancer world
- ✓Limitation and weakness point of this knowledge is still under observation
- ✓Also we still need more aptamer in order to diagnose more and specific illness.

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Thank you